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(54) [Title Of The Invention] Silica Particle Composition For
Extraction Of Nucleic Acid Or Protein

(57) [Abstract]

[Subject] The invention offers a silica particle composition dispersed to a high degree in an aqueous solution, and a reagent that easily extracts nucleic acid or protein by using the said silica particle composition.

[Means] Silica particle composition suspended in a salt solution containing alkali metal, alkali earth metal or halide of ammonia and sodium acetate or sodium thiocyanate where the silica particles are at least 0.5M, and/or suspended in a saccharide solution that is a solution of monosaccharide, polysaccharide or sugar alcohol that is at least 40 (W/V)%; a method of pouring the silica particle composition from a reagent vessel into a reaction vessel using the said silica particle composition in a pouring tool or pouring device; a reagent kit for extracting nucleic acid or protein contained in the said silica particle composition; and, a method of extracting the nucleic acid or protein.

[Scope Of Patent Claim]

[Claim Paragraph 1] Silica particle composition for extraction of nucleic acid or protein characterized in that silica particles are suspended in a salt solution of inorganic salts or organic salts where they are at least 0.5 M and/or in a saccharide solution of monosaccharide, polysaccharide or sugar alcohol where they are at least 40 (W/V)%.

[Claim Paragraph 2] Silica particle composition described in Claim Paragraph 1 where the silica particles are a complex of silicon dioxide crystals, silicon oxide crystals other than silicon dioxide crystals, diatomaceous earth, glass powder, chemically modified silica or silica combined with superparamagnetic metal oxides.

[Claim Paragraph 3] Silica particle composition described in Claim Paragraph 1 where the specific gravity of the silica particles is 1.0 to 1.8.

[Claim Paragraph 4] Silica particle composition described in Claim Paragraph 1 where the inorganic salt or organic salt solution is a solution containing alkali metal, alkali earth metal or ammonia halide, and sodium acetate or sodium thiocyanate.

[Claim Paragraph 5] Silica particle composition described in Claim Paragraph 1 where the inorganic salt or organic salt solution is a salt solution selected from among a group comprising sodium chloride, sodium iodide, lithium chloride, lithium iodide, magnesium chloride, ammonium acetate and sodium thiocyanate.

[Claim Paragraph 6] Silica particle composition described in Claim Paragraph 1 where the saccharide solution is a saccharide solution selected from among a group comprising glucose, saccharose, sorbitol or mannitol.

[Claim Paragraph 7] Silica particle composition for extracting nucleic acid or protein characterized in that silica particles--in a complex of superparamagnetic metal oxides with silicon dioxide crystals, silicon oxide crystals other than silicon dioxide crystals, diatomaceous earth, glass powder, chemically modified silica or silica with the specific gravity being 1.0 to 1.8 and particle diameters being 0.1 to 100 μm --are suspended in a solution containing alkali metal, alkali earth metal or ammonia halide and sodium acetate or sodium thiocyanate that are at least 0.5 M an/or suspended in a saccharide solution of monosaccharide, polysaccharide or sugar alcohol that is at least 40 (W/V)%.

[Claim Paragraph 8] Method of pouring a silica particle solution characterized in that it uses a pouring tool or a pouring implement to pour the silica particle compositions of any 1 claim among Claim Paragraphs 1 to 7 from a reagent vessel to a reaction vessel.

[Claim Paragraph 9] Reagent kit used to extract nucleic acid or protein containing any of the silica particle compositions described in Claim Paragraphs 1 through 7, an adsorption reagent, a reagent for washing and a reagent for elution.

[Claim Paragraph 10] Reagent kit used to extract nucleic acid or protein described in Claim Paragraph 9 where reagent for adsorption contains a kaotropic reagent, a buffer solution and as required an organic solvent.

[Claim Paragraph 11] Method of extracting nucleic acid or protein characterized in that cells containing protein or nucleic acid are mixed with any of the silica particle compositions described in Claim Paragraphs 1 through 7 and with a reagent for adsorption, the protein and nucleic acid are extracted from the said cells, the nucleic acid and protein are bonded to the said silica particles, the residual ingredients are separated, and after washing the said silica particles, the nucleic acid and protein bonded to the said silica particles are eluted.

[Detailed Explanation Of The Invention]

[0001]

[Industrial Field To Which The Invention Belongs] The present invention relates to a silica particle composition capable of being stably dispersed in aqueous solutions, and in further detail relates to a silica particle composition suspended in a salt solution at comparatively high concentrations and/or in a saccharide solution at high concentrations, and further relates to a method of pouring from a reagent vessel holding the said composition into a reaction vessel such as a reagent tube, microtube or plate, and to a reagent kit for extracting nucleic acid or protein containing the said composition, and to a method of extracting nucleic acid and

protein. The composition of the present invention can be applied for example in an automatic nucleic acid extraction apparatus that isolates nucleic acid utilizing the bonding of the nucleic acid onto silica particles.

[0002]

[Prior Technology] In recent years within the field of molecular chemistry, there have been many investigations carried out to separate such as deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) from biological specimens and analyze them. Various methods have been considered for separating the DNA and RNA from the biological specimens, among which the method of bonding (adsorbing) the nucleic acid of such as DNA and RNA physically or specifically to the molecular surfaces of such as silica (as in Japanese Patent Disclosure Hei 2-289596) seems to be much in use because of its simplicity. However, particles with heavy specific gravities such as silica particles have poor dispersibility in aqueous solutions, leading to a situation where the operation of pouring the said particles from the reagent vessel to such as a microtube is necessarily one of particular difficulty.

[0003] Methods have been devised to separate magnetic particles using magnetic force in order to automate these processes (as Japanese Patent Hei 4-501959, Japanese Patent Hei 5-504095, Japanese Early Disclosure Hei 8-32027), and particularly in cases when complexes of particles such as silica and

superparamagnetic metal oxides were used, more pouring operations were required because they were heavier than conventional silica particles and their dispersibility was lower. Consequently, under prior technology where magnetic silica particles suspended in water were poured from a reagent vessel into such as a microtube, it was necessary to stir the said particle aqueous solution vigorously and then complete the pouring in the short time period while the particles remained dispersed. Also, nonuniformities in concentration occurred easily in the solutions thus poured and there were many differences in particle concentrations because of the pouring sequences, making them unsuitable for quantitative analyses.

[0004]

[Problems To Be Resolved By The Invention] Accordingly there have been fervent hopes for being able to disperse heavy specific gravity silica particles in aqueous solutions for long periods of time, from the standpoints of easy pouring of the said particles from their reagent vessels to reaction vessels, and of pouring purity. The greatest hope was to use pouring apparatus for pouring of particle complexes of such as silica and superparamagnetic metal oxides into test tubes, microtubes or plates. An object of the present invention lies in offering a silica particle composition capable of a high degree of dispersion in aqueous solutions.

[0005]

[Means For Resolving The Problems] The present inventors have carried out various investigations into dispersing heavy specific gravity silica particles in aqueous solutions, focusing on the compositions of the aqueous solutions and their concentrations. As a result, they discovered that it is possible to use solutions containing high concentrations of salts such as sodium chloride, sodium iodide, lithium chloride and ammonium acetate and/or saccharides such as saccharose and sorbitol to put heavy specific gravity silica particles into a dispersed state for long periods of time, and thus they perfected the present invention.

[0006] That is, the present invention is a silica particle composition for extraction of nucleic acid or protein characterized in that the silica particles are suspended in a salt solution of inorganic or organic salts that are at least 0.5 M and/or a saccharide solution comprising monosaccharides, polysaccharides or sugar alcohols that are at least 40 (W/V)%.

[0007] Also, the present invention is a method of pouring silica particle solutions characterized in that a pouring implement or device is used to pour the said silica particle compositions from reagent vessels to reaction vessels.

[0008] Further, the present invention is a reagent kit for extracting nucleic acid or protein characterized in that it contains the said silica particle composition, a reagent for adsorption, a reagent for washing and a reagent for elution.

[0009] The present invention is a method of extracting nucleic acid or protein characterized in that it mixes cells containing protein and nucleic acid with the said silica particle composition and adsorption reagent, removes protein and nucleic acid from the said cells, bonds the said nucleic acid or protein to the said silica particles, separates the residual ingredients, washes the said silica particles and finally elutes the said nucleic acid or protein bonded to the said silica particles.

[0010]

[Modes Of Working The Present Invention] Within the present invention, what are called silica particles are particles that contain silica, for example such as silicon dioxide crystals, other silicon oxides, diatomaceous earth, glass powder, chemically modified silica, and complexes of silica with superparamagnetic metal oxides.

[0011] Silica particles constructed from these ingredients are substances having specific gravities heavier than water, ordinarily with specific gravities of 1.0 to 1.8, they precipitate when suspended in water to form hard pellets on the bottoms of vessels, and are ordinarily difficult to keep in a suspended state in aqueous solutions for long periods of time. Particles used for nucleic acid adsorption in the field of molecular biology in particular often have diameters on the order of about 0.1 to 100 μm , and many of them have these properties.

[0012] The silica particle composition of the present invention is one that disperses silica particles having such properties in aqueous solutions at high concentrations. It is easy to predict that it will be possible to apply this technology to particles other than silica particles, such as latex particles, metal particles and modified metal particles.

[0013] Examples of superparamagnetic metal oxides that may be offered are iron oxide (Fe_3O_4). An example of complexes of silica with the said superparamagnetic metal oxides are iron oxide (Fe_3O_4) added to an alcohol solution of tetraethoxysilane, where silica is deposited on the surface of the iron oxide (Fe_3O_4) under superparasonic wave dispersion. The dispersion thus obtained receives an addition of sodium silicate and is then emulsified with additions of organic solvent and surfactant (a toluene solution of sorbitan monostearate), to form a W/O type emulsion. An ammonium sulfate aqueous solution is added to this emulsion, followed by sufficient stirring. After this, it is separated by filtration, washed in water, precipitated with alcohol and dried to obtain the required globular silica particles.

[0014] To offer an example of a complex of silica and superparamagnetic metal oxide: (1) the magnetic particles include superparamagnetic iron oxide, (2) the specific surface area is 100 to $800 \text{ m}^2/\text{g}$, (3) the said iron oxide is coated with silica, (4) it is further complexed with an inorganic porous wall substance constructed of microfine particles, (5) the weight of the said iron

oxide is 10 to 60 wt%, (6) the surface area pore diameters of the magnetic silica particles are 0.1 to 1.5 ml/g, and (8) the particle diameters of the silica particles are 0.1 to 100 and preferably 0.5 to 15 μm .

[0015] Such a complex of silica and the said superparamagnetic metal oxide is exemplified as containing magnetic silica particles made by Suzuki Yushi [Suzuki Oil and Fat] and 30 (W/V)% iron oxide (Fe_3O_4), (particle diameters 1 to 10 μm , specific surface area 280 m^2/g , surface pore diameters 2 to 6 nm, and pore volume 0.025 ml/g).

[0016] The salt solution used with the present invention is an inorganic solution or organic solution, the inorganic salt solutions being solutions including alkali metals, alkali earth metals or ammonia halides or thiocyanate, for example aqueous solutions of sodium chloride, sodium iodide, lithium chloride, lithium iodide, magnesium chloride and sodium thiocyanate. The organic salt solutions are solutions including alkali metals, alkali earth metals or acetates of ammonia, for example aqueous solutions of sodium acetate, lithium acetate and magnesium acetate. These may also be used in combinations. Their concentrations will be at least 0.5 M, and preferably will be high concentrations of 3 to 10 M. At less than 0.5 M, the dispersibility of the silica particles drops, and pouring purity declines.

[0017] The said solutions may also contain other ingredients, for example such as buffer solutions, chelating agents or surfactants.

[0018] The saccharide solutions used with the present invention are solutions of monosaccharides, polysaccharides or sugar alcohols, for example including monosaccharides such as glucose, galactose and fructose, disaccharides such as saccharose, maltose and lactose, and sugar alcohols such as sorbitol, manitol and galacitol. These may also be used in combinations. The concentration of saccharides with the present invention will be at least 40 (W/V)%, and preferably 50 to 60 (W/V)%. At less than 40 (W/V)%, the dispersibility of the silica particles drops, and there tends to be insufficient dispersion making it difficult to obtain satisfactory pouring purity. The said solutions may also contain other ingredients, for example such as preservatives, buffer solutions, chelating agents or surfactants.

[0019] In regard to the dispersibility of the silica particles in aqueous solutions, the types and concentrations of substances dissolved in the solutions are important, and as a result there is a tendency for them to be high in salts such as sodium chloride, sodium iodide, lithium chloride, lithium iodide, magnesium chloride and ammonium acetate, or in saccharides such as saccharose and sorbitol. The same effect may be expected salts and saccharides other than these. With the present invention, saccharides may also be dissolved in salt solutions.

[0020] In the field of automatic pouring of the said silica particles from reagent vessels into such as microtubes that will be the reaction vessels, there is mainly an operation of pouring the suspended silica particles within about 5 minutes, making it important for dispersibility of the particles to last for at least 5 minutes. When the silica particle composition of the present invention is used, particle dispersibility increases markedly within the 5 minute period compared to silica particles suspended in water, and it is clear that pouring purity rises, and it is possible to construct an automatic pouring system for applying the method of the present invention. Also, when the silica particle composition of the present invention is used, it is found that the particle dispersibility increases markedly after 5 minutes compared to ordinary suspensions in distilled water, and it is thought that it is also easy to resuspend precipitated particles.

[0021] Considering the point of solution decay over long storage periods, salt solutions are preferred for suspensions of silica particles. But when saccharide solutions are used, preservatives should be added. Among salt solutions, the preferred are salt solutions such as sodium chloride, lithium chloride and magnesium chloride. From the standpoint of salt crystal deposition, lithium chloride in particular is most unlikely to form crystals and is therefore superior.

[0022] In working the present invention, the said salt solutions of inorganic salts or organic salts and/or saccharide

solutions of monosaccharides, polysaccharides or sugar alcohols are used in a reaction system such as nucleic acid adsorption, and the types of solutions should be selected so that no damage is done to the said reaction systems. Particularly when adsorbing nucleic acid onto the silica particles, it is necessary to select solutions that will not affect the selectability and allowability of the types and concentrations of ions used in the reaction system.

[0023] The silica particle solutions following the present invention can maintain the dispersed states of particles such as heavy specific gravity silica in aqueous solutions over comparatively long time periods, and make it easy to pour from particle reagent vessels into such as test tubes, microtubes and plates serving as reaction vessels, particularly with automatic pouring equipment. The silica particle compositions of the present invention are used for such as nucleic acid extraction apparatus that utilize adsorption of the nucleic acid onto silica particles. But it is also possible to apply them to various applications that utilize silica particles, for example in fields such as cosmetic materials, paints and adhesives.

[0024] One mode of working the present invention is with a composition that suspends a complex of particles of such as silica and superparamagnetic metal oxides (magnetic silica particles: diameters 1 to 10 μm) in about 0.5 to 5 M of sodium chloride or lithium chloride. By using this composition, it becomes possible to supply a stabilized particle suspension and have automatic pouring

of the magnetic silica particles, so that it becomes possible to apply it to such as apparatus for automatic extraction of nucleic acid.

[0025] The silica particle composition of the present invention will also pour from a reagent vessel into such as a test tube, microtube or plate serving as the reaction vessel, using a pouring tool or pouring device. Pouring tools that may be considered are such as measuring pipettes, micropipettes and syringes. Pouring devices that may be considered are such as pipette type pouring devices using cylinders and pressure type pourers using pumps. Pouring methods to be considered are methods of pouring suspended solutions after inserting a cylinder inside the tube and methods of using the pressure of such as a pump in the tube to extrude from a nozzle.

[0026] The reagent kit for nucleic acid or protein extraction of the present invention includes the said silica particle composition, an adsorption reagent for easily adsorbing nucleic acid or protein, a washing reagent for washing unspecified ingredients adsorbed to the particles and an elution reagent for eluting the nucleic acid or protein from the particle surfaces. Adsorption reagents may be exemplified by kaotropic reagents such as guanidine salts, sodium iodide, potassium iodide, sodium thiocyanate (iso), urea or mixtures of these. Adsorption reagents may also contain buffers such as acetate buffer solutions, citric acid buffer solutions and tris buffer solutions, chelating agents such as ethylenediaminetetracetic acid tetrasodium salt (EDTA)

and/or organic solvents such as ethanol. Washing reagents may be ones that contain the above tropic substances or organic solvents such as ethanol. The washing reagents may also contain the said buffers and/or chelating agents. Low salt concentration buffer solutions and water may be used in the elution reagents that elute the nucleic acid or proteins from the molecule surfaces. Ingredients in the adsorption solutions, washing solutions and elution solutions other than stated above can be selected in various combinations depending on the application.

[0027] When extracting nucleic acid and proteins, it is necessary to carry out operations such as mixing, separation and heating of the reagents contained in the said reagent kit in a prescribed order. For example, cells containing proteins and nucleic acid are mixed with the said silica particle composition and a reagent for adsorbing nucleic acid or proteins, the proteins and nucleic acid are extracted from the said cells, the proteins and nucleic acid are mixed with the said silica particles, the residual ingredients are separated, and after washing the said silica particles, the nucleic acid or proteins bonded to the silica particles are eluted to extract the nucleic acid or proteins. The eluted nucleic acid can be detected by a known detection method after amplification using a known nucleic acid amplification method. The method of extracting nucleic acid or protein of the present invention can be suitably used in preparing nucleic acid or protein for use in research or in clinical diagnostics.

[0028]

[Examples] The present invention will next be explained specifically by means of examples.

Example 1 Particle Precipitation Tests

(1) Preparing Magnetic Silica Particle Suspensions

Silica particle suspensions were prepared by making a suspension of 0.4 g of magnetic silica particles (particle diameters 1 to 10 μm , containing 30% (W/V) of iron oxide (Fe_3O_4), specific surface area 280 m^2/g , surface pore diameters 2 to 6 nm, pore volume 0.025 ml/g: made by Suzuki Jushi [Suzuki Oil and Fat]) in microtubes holding 1 ml solutions containing the compounds shown below in Table 1. The reagents used in preparing the reagents below were made by NAKARAITSUKU Company, and the water used was distilled water.

[0029] (2) Precipitation Tests

The microtubes containing the respective magnetic silica particle suspensions prepared in (1) were mixed by tumbling for complete suspensions, and were taken in up to a scale of 1 ml with 1 ml syringes (made by TEREMO Company, SS-01T). Then the syringes were attached to standing test tubes, and after 5 minutes the interfaces between the solution and the magnetic silica particles were measured for height, that is the height of the lines up to which a number of ml of particles precipitated. Measurements were made 2 times, and the average interface heights were divided by the interface heights at zero minutes to

obtain the dispersion rates. The results are also shown in Table 1.

[0030]

[Table 1]

Dispersion Rates After Magnetic Silica Particle Suspensions Were
Let Stand For 5 Minutes

Samples	Dispersion Rates
5 M sodium chloride solution	0.95
5 M sodium iodide solution	0.99
5 M lithium chloride solution	0.97
4 M lithium chloride solution	0.94
3 M lithium chloride solution	0.94
2 M lithium chloride solution	0.94
1 M lithium chloride solution	0.93
0.5 M lithium chloride solution	0.90
0.2 M lithium chloride solution	0.88
5 M ammonium acetate solution	0.96
2 M magnesium chloride solution	0.95
2 M potassium chloride solution	0.85
5 M guanidine hydrochloride	0.89
50% (W/V) saccharose solution	1.00
40% (W/V) saccharose solution	0.91
25% (W/V) saccharose solution	0.83
50% (W/V) sorbitol solution	0.99
50% (W/V) glycerin solution	0.88
Distilled water	0.70

[0031] As will be clear from Table 1, high particle dispersion effects were found in salt solutions such as sodium chloride, sodium iodide, lithium chloride, ammonium chloride and magnesium chloride, and in saccharose solutions such as saccharose and sorbitol. These solutions maintained interfaces of 90% or more compared to the distilled water solution whose interface fell to 70% after 5 minutes. This trend becomes more marked as solution concentrations became thicker, as will be understood after seeing that the 5 M sodium chloride solution, sodium iodide solution, lithium chloride solution, ammonium chloride solution and the 2 M magnesium chloride solution gave dispersion rates of 0.95 or more.

[0032] In regard to salt concentration, the lithium chloride was conspicuous at 0.5 M and over, and even at 0.5 M its dispersibility was clearly higher than the high specific gravity 2 M potassium chloride solution. In regard to saccharide solution concentrations, the saccharose was outstanding at concentrations of 40% (W/V) or more. From these facts, it is clear that the effect of raising particle dispersibility is largely governed by the types of substances dissolved in the solutions and by their concentrations.

[0033] The 5 M lithium chloride solution was compared with the distilled water for particle dispersion rates after 5 minutes and the difference was still conspicuous even after 30 minutes, and while the dispersion rate went to 0.4 or less in the distilled water

solution, it was 0.6 or more in the 5M lithium chloride suspension (see Fig. 1).

[0034] Example 2 Pouring Tests

(1) Preparation Of Particle Suspensions

Following the same method as in Example 1, 2 ml magnetic silica particle suspensions (5 M lithium chloride solution, distilled water) were prepared.

[0035] 1.2 ml of magnetic silica particle suspensions of previously prepared 5 M lithium chloride in distilled water were taken into continuous pouring pipettes (made by EPPENDORUFU) that were fitted with 2.5 m capacity KONBICHIPPU's (made by EPPENDORUFU), and 19 of these were continuously poured for 5 seconds each and 50 μ l each in previously weight microtubes. At time of pouring, the pouring device was perpendicular to the bottom surface. After pouring was completed, the pour amounts were checked for near accuracy, the poured microtubes were weighed, and the previously weighed values were subtracted to calculate the weights of the poured particle solutions.

[0036] These results are shown in Fig. 2. As will be apparent from Fig. 2, the pouring of magnetic silica particles suspended in 5 M lithium chloride as compared to the pouring of the suspension in distilled water gave more stable numerical values. The pour weight CV (%) of the lithium chloride suspension and the distilled water suspension (standard

variation/average value $\times 100$)) were respectively 0.94% and 3.26%. This difference will be inferred as being based on the concentration of magnetic particles contained in the poured suspensions.

[0037] When continuously pouring suspensions in distilled water, the weight of the liquid poured gradually increases from the first up to the 13th time, and after the 13th time, shows a tendency to decrease gradually. This phenomenon can easily be anticipated from the results of Example 1, and it may be expected that a concentration slope forms in the particle suspension. Thus, by using the method of the present invention, it becomes possible to pour a uniform silica suspension continuously.

[0038]

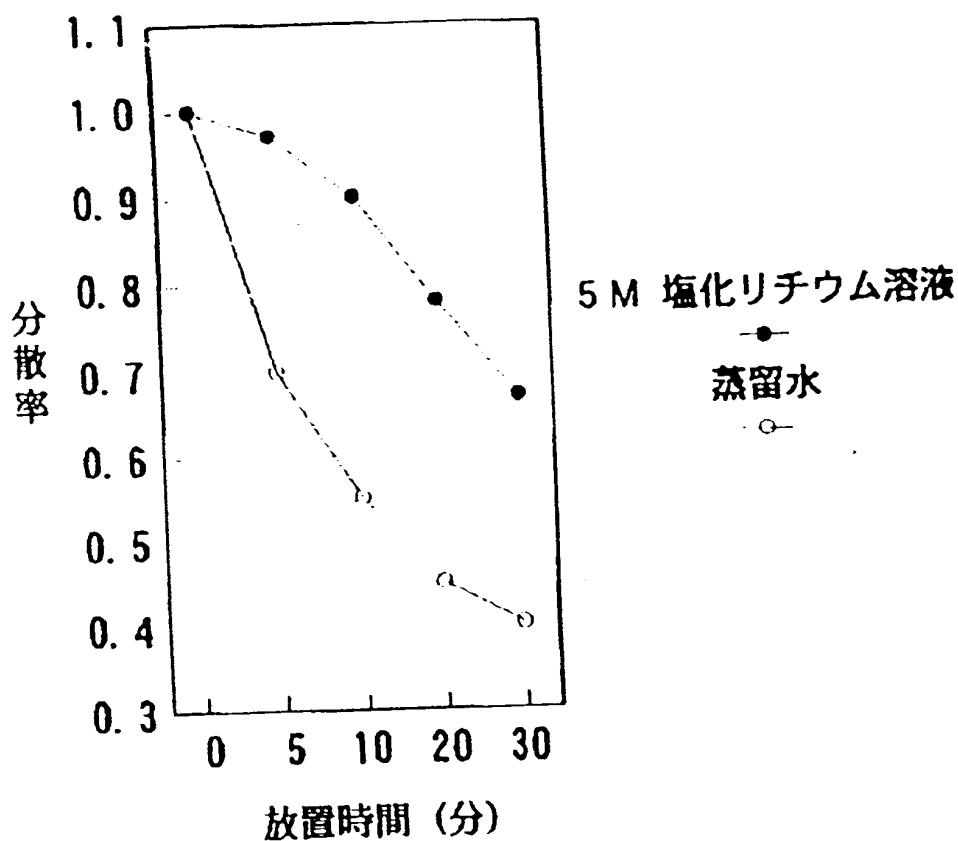
[Effect Of The Invention] By means of the present invention, it becomes possible to have silica particles of high specific gravity easily dispersed in aqueous solutions. The silica particle composition of the present invention can also be applied in such as nucleic acid extraction apparatus employing automatic pouring devices. Application of this method to purification of nucleic acid and protein can give a more reliable and reproducible extraction effect than prior methods.

[Brief Explanation Of The Drawings]

[Fig. 1] This is a diagram showing the relation between the dispersion rate of a magnetic silica particle suspension and the time let stand.

[Fig. 2] This is a diagram showing variations in pouring weight at times when magnetic silica particle suspensions are continuously poured.

[Fig. 1]



{Left:} Dispersion Rate

{Bottom:} Time Left Standing (minutes)

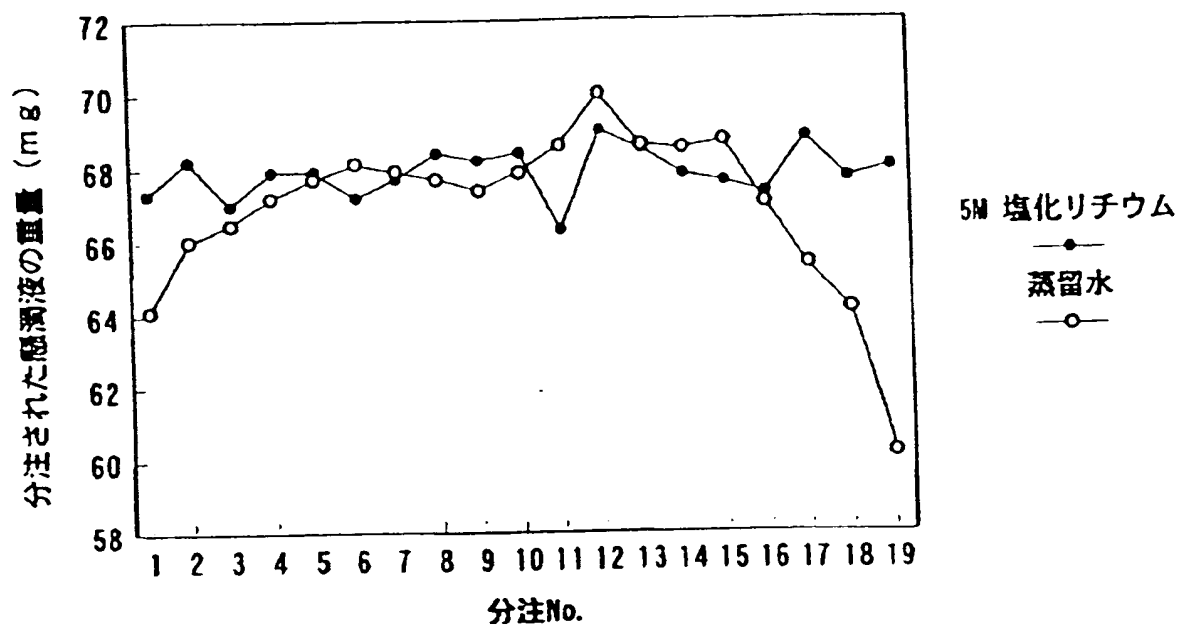
{Right:} 5M Lithium Chloride Solution

Distilled Water

- • -

- ○ -

[Fig. 2]



{Left:} Weight Of Poured Suspension (mg)

{Bottom:} Pour No.

{Right:} 5M Lithium Chloride - • -

Distilled Water - o -

{Start New Document}

[Procedural Revision]

[Date Presented] June 2, 1997

[Procedural Revision 1]

[Object Of Revision] Specification

[Object Of Revision Portion] Scope Of Patent Claim

[Method Of Revision] Change

[Content Of Revision]

[Scope Of Patent Claim]

[Claim Paragraph 1] Silica particle composition for extraction of nucleic acid or protein characterized in that silica particles are suspended in a salt solution of inorganic salts or organic salts where they are at least 0.5 M and/or in a saccharide solution of monosaccharide, polysaccharide or sugar alcohol where they are at least 40 (W/V)%.

[Claim Paragraph 2] Silica particle composition described in Claim Paragraph 1 where the silica particles are a complex of silicon dioxide crystals, silicon oxide crystals other than silicon dioxide crystals, diatomaceous earth, glass powder, chemically modified silica or silica combined with superparamagnetic metal oxides.

[Claim Paragraph 3] Silica particle composition described in Claim Paragraph 1 where the inorganic salt or organic salt solution is a solution containing alkali metal, alkali earth metal or ammonia halide, and sodium acetate or sodium thiocyanate.

[Claim Paragraph 4] Silica particle composition described in Claim Paragraph 1 where the inorganic salt or organic salt solution is a salt solution selected from among a group comprising sodium chloride, sodium iodide, lithium chloride, lithium iodide, magnesium chloride, ammonium acetate and sodium thiocyanate.

[Claim Paragraph 5] Silica particle composition described in Claim Paragraph 1 where the saccharide solution is a saccharide solution selected from among a group comprising glucose, saccharose, sorbitol or mannitol.

[Claim Paragraph 6] Silica particle composition for extracting nucleic acid or protein characterized in that silica particles--in a complex of superparamagnetic metal oxides with silicon dioxide crystals, silicon oxide crystals other than silicon dioxide crystals, diatomaceous earth, glass powder, chemically modified silica or silica with the specific gravity being 1.0 to 1.8 and particle diameters being 0.1 to 100 μm --are suspended in a solution containing alkali metal, alkali earth metal or ammonia halide and sodium acetate or sodium thiocyanate that are at least 0.5 M an/or suspended in a saccharide solution of monosaccharide, polysaccharide or sugar alcohol that is at least 40 (W/V)%.

[Claim Paragraph 7] Method of pouring a silica particle solution characterized in that it uses a pouring tool or a pouring implement to pour the silica particle compositions of any 1 claim among Claim Paragraphs 1 to 6 from a reagent vessel to a reaction vessel.

[Claim Paragraph 8] Reagent kit used to extract nucleic acid or protein containing any of the silica particle compositions described in Claim Paragraphs 1 through 6, an adsorption reagent, a reagent for washing and a reagent for elution.

[Claim Paragraph 9] Reagent kit used to extract nucleic acid or protein described in Claim Paragraph 9 [sic] where reagent for adsorption contains a kaotropic reagent, a buffer solution and as required an organic solvent.

[Claim Paragraph 10] Method of extracting nucleic acid or protein characterized in that cells containing protein or nucleic acid are mixed with any of the silica particle compositions described in Claim Paragraphs 1 through 7 and with a reagent for adsorption, the protein and nucleic acid are extracted from the said cells, the nucleic acid and protein are bonded to the said silica particles, the residual ingredients are separated, and after washing the said silica particles, the nucleic acid and protein bonded to the said silica particles are eluted.

[Procedural Revision 2]

[Object Of Revision]	Specification
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[Object Of Revision Portion]	0011
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[Method Of Revision]	Change
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[Content Of Revision]

[0011] Silica particles constructed from these ingredients are substances having specific gravities heavier than water, they precipitate when suspended in water to form hard pellets on the

bottoms of vessels, and are ordinarily difficult to keep in a suspended state in aqueous solutions for long periods of time. Particles used for nucleic acid adsorption in the field of molecular biology in particular often have diameters on the order of about 0.1 to 100 μm , and many of them have these properties.

End.

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最終頁に続く

(54) 【発明の名称】 核酸またはタンパク質抽出用シリカ粒子組成物

(57) 【要約】

【課題】 水溶液中に高度に分散されたシリカ粒子組成物および該組成物を使用して核酸またはタンパク質を簡便に抽出する試薬を提供する。

【解決手段】 シリカ粒子が、少なくとも0.5Mであるアルカリ金属、アルカリ土類金属またはアンモニアのハロゲン化物、酢酸塩またはチオシアン酸塩を含む塩溶液および/または少なくとも10(w/v)%である単糖類、多糖類または糖アルコールの溶液である糖類溶液に懸濁されたシリカ粒子組成物および該シリカ粒子組成物を分注器具または分注機器を用いて、試薬容器から反応容器へ分注するシリカ粒子溶液を分注する方法、該シリカ粒子組成物を含有する核酸またはタンパク質抽出用試薬キットならびに核酸またはタンパク質抽出法。

【特許請求の範囲】

【請求項1】 シリカ粒子が、少なくとも0.5Mである無機塩または有機塩の塩溶液および、または少なくとも4.0(w/v)%である単糖類、多糖類または糖アルコールの糖類溶液に懸濁されていることを特徴とする核酸またはタンパク質抽出用シリカ粒子組成物。

【請求項2】 シリカ粒子が、二酸化珪素結晶、二酸化珪素結晶以外の珪素酸化物、珪藻土、ガラス粉末、化学修飾シリカまたはシリカと超常磁性金属酸化物との複合体である請求項1に記載のシリカ粒子組成物。

【請求項3】 シリカ粒子の比重が1.0~1.8である請求項1に記載のシリカ粒子組成物。

【請求項4】 無機塩または有機塩溶液が、アルカリ金属、アルカリ土類金属またはアンモニアのハロゲン化物、酢酸塩またはチオシアン酸塩を含む溶液である請求項1に記載のシリカ粒子組成物。

【請求項5】 無機塩または有機塩溶液が、塩化ナトリウム、ヨウ化ナトリウム、塩化リチウム、ヨウ化リチウム、塩化マグネシウム、酢酸アンモニウムおよびチオシアン酸ナトリウムよりなる群から選択された塩溶液である請求項1に記載のシリカ粒子組成物。

【請求項6】 糖類溶液がグルコース、サッカロース、ソルビトールおよびマンニトールよりなる群から選択された糖類溶液である請求項1に記載のシリカ粒子組成物。

【請求項7】 比重が1.0~1.8であって、粒径が0.1~1000nmである二酸化珪素結晶、二酸化珪素結晶以外の珪素酸化物、珪藻土、ガラス粉末、化学修飾シリカまたはシリカと超常磁性金属酸化物との複合体であるシリカ粒子が、少なくとも0.5Mであるアルカリ金属、アルカリ土類金属またはアンモニアのハロゲン化物、酢酸塩またはチオシアン酸塩を含む溶液および、または少なくとも4.0(w/v)%である単糖類、多糖類または糖アルコールの糖類溶液に懸濁されていることを特徴とする核酸またはタンパク質抽出用シリカ粒子組成物。

【請求項8】 分注器具または分注機器を用いて、請求項1~7のいずれか1項記載のシリカ粒子組成物を試薬容器から反応容器へ分注することを特徴とするシリカ粒子溶液を分注する方法。

【請求項9】 請求項1~7のいずれか1項記載のシリカ粒子組成物、吸着用試薬、洗浄用試薬および溶出用試薬を含有する核酸またはタンパク質抽出用試薬キット。

【請求項10】 吸着用試薬が、カオトロピックス試薬、緩衝液および必要により有機溶媒を含有する請求項9記載の核酸またはタンパク質抽出用試薬キット。

【請求項11】 タンパク質および核酸を含む細胞を請求項1~7のいずれか1項記載のシリカ粒子組成物および吸着用試薬と混合して、該細胞からタンパク質および核酸を取り出し、該シリカ粒子に核酸またはタンパク質を結合させて、残余の成分と分離させ、該シリカ粒子を

洗浄した後、該シリカ粒子に結合した核酸またはタンパク質を溶出させることを特徴とする核酸またはタンパク質抽出法。

【発明の詳細な説明】

【0001】

【発明が属する技術分野】本発明は、水溶液中に安定に分散させ得るシリカ粒子組成物に関し、さらに詳しくは、比較的高濃度の塩溶液および、または高濃度の糖類溶液中に懸濁させたシリカ粒子組成物に関し、さらに、該組成物の試薬容器から反応容器となる試験管、マイクロチューブまたはプレート等への分注方法ならびに該組成物を含有する核酸またはタンパク質抽出用試薬キットおよび核酸またはタンパク質抽出法に関する。本発明の組成物は、例えばシリカ粒子への核酸の結合を利用して核酸を単離するような核酸抽出試薬を用いた自動核酸抽出装置にも応用し得る。

【0002】

【従来の技術】近年、分子生物学の分野において、生体試料よりデオキシリボ核酸(DNA)やリボ核酸(RNA)などを分離して、これらを解析する研究が盛んに行われている。生体試料よりDNAやRNAを分離するにあたり、様々な方法が考案されてきたが、その中でもシリカなどの粒子表面に物理的または特異的にDNAやRNAなどの核酸を結合(吸着)させる方法(特開平2-289596号公報など)が、その簡便さから盛んに使用されるようになりつつある。一方、シリカ粒子など比重の重い粒子は水溶液中では分散性が悪く、該粒子を試薬容器からマイクロチューブ等へ分注する操作は、特に煩雑とならざるをえない状況にある。

【0003】これらの操作を自動化するにあたり、磁性体粒子を磁力を用いて分離する方法が考案されている(特表平4-501959号公報、特表平5-504095号公報、特開平8-32027号公報など)が、特にシリカと超常磁性金属酸化物などの複合体粒子を用いた場合、通常のシリカ粒子に比べて重く、分散性が低いため、分注操作はさらに煩雑なものにならざるを得ない。したがって、従来の技術、すなわち、水に懸濁された磁性シリカ粒子を試薬容器からマイクロチューブ等へ分注する場合、該粒子水溶液を激しく攪拌した後、粒子が分散している短時間に分注を完了しなければならない。また、そのようにして分注した溶液は濃度にムラができやすく、分注の順番により粒子の濃度が異なることが多いため、特に定量的な解析には向かないものであった。

【0004】

【発明が解決しようとする課題】そこで、比重の重いシリカ粒子を長時間、水溶液中に分散させておくことが、該粒子の試薬容器から反応容器への分注の簡便さ、また、分注精度の点からも強く望まれていた。特に、自動分注機を用いてシリカと超常磁性金属酸化物などの複合体粒子を反応容器となる試験管、マイクロチューブまた

はフレート等へ分注するに当たっては最大の命題であった。すなわち、本発明の目的は、水溶液中に高度に分散させるシリカ粒子組成物を提供することである。

【0005】

【課題を解決するための手段】本発明者らは比重の重いシリカ粒子を、水溶液中に分散させるにあたり、水溶液の組成およびその濃度に着目し、種々の検討を行った。その結果、塩化ナトリウム、ヨウ化ナトリウム、塩化リチウム、酢酸アンモニウムなどの塩および、またはサッカロース、ソルビトールなどの糖類を高濃度で含む溶液が、比重の重いシリカ粒子を比較的時間、分散状態

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の糖アルコール類が含まれる。これらを組み合わせても良い。本発明では糖類の濃度が少なくとも40(w/v) %、好ましくは50~60(w/v) %である。40(w/v) %未満であると、シリカ粒子の分散性が低下し、十分に分散されず満足いく分注精度を得ることが難しくなる傾向にある。上記溶液は他の成分、例えば防腐剤、緩衝液、キレート剤または界面活性剤などを含有しても良い。

【0019】シリカ粒子の水溶液中での分散性は、溶液中に溶解している物質の種類及び濃度が重要であり、その効果は、塩化ナトリウム、ヨウ化ナトリウム、塩化リチウム、ヨウ化リチウム、塩化マグネシウムおよび酢酸アンモニウムなどの塩、またはサッカロース、ソルビトールなどの糖類に高い傾向が認められる。これら以外の塩類および糖類においても同様な効果が期待される。本発明では塩溶液に糖類が溶解されていてもよい。

【0020】該シリカ粒子を試薬容器から反応容器となるマイクロチューブ等へ自動分注する分野では、一般的には懸濁したシリカ粒子を約5分間以内に分注する操作が主体となるため、少なくとも5分間以内での粒子の分散性が重要となる。本発明のシリカ粒子組成物を用いた場合、シリカ粒子を水に懸濁した場合に比べ、5分間以内での粒子の分散性は飛躍的に向上し、分注の精度が上昇することが明らかとなり、この方法に応用した自動分注システムの構築も可能である。また、本発明のシリカ粒子組成物を用いると、5分間以後の粒子の分散性も蒸留水に懸濁した場合に比べ、飛躍的に向上していることも確認されており、沈殿した粒子の再懸濁も容易になるものと思われる。

【0021】長期保存における溶液の腐敗という面から考えると、シリカ粒子の懸濁には、塩類の溶液が好ましい。一方、糖類溶液を用いる場合は、防腐剤を添加することが好ましい。また、塩溶液としては、塩化ナトリウム、塩化リチウム、塩化マグネシウムなどの塩溶液が好ましい。また、塩結晶の析出などのことも考えると、特に塩化リチウムが最も結晶が生じにくく、優れている。

【0022】本発明を実施するに当たり、上記無機塩または有機塩の塩溶液および、または単糖類、多糖類または糖アルコールの糖類溶液を核酸吸着等の反応系に持ち込むことによって、該反応系が阻害されないように、溶液の種類を選択することが好ましい。特に、核酸等をシリカ粒子に吸着させるような場合、反応系に持ち込んだイオンの種類や濃度により、その選択性や許容力などに影響が及ばないような溶液を選択する必要がある。

【0023】本発明によるシリカ粒子組成物は、比重の重いシリカなどの粒子を比較的長時間にわたって、水溶液中に分散状態に保つことができ、粒子の試薬容器から反応容器となる試験管、マイクロチューブおよびプレート等への分注（特に自動分注機）を用いた分注を容易にする。本発明のシリカ粒子組成物は、シリカ粒子への核

酸の吸着を利用した核酸の抽出装置などに使用される。また、シリカ粒子を利用した様々な用途、例えば化粧材料、塗料、接着などの分野への応用も可能である。

【0024】本発明の一実施態様としては、シリカと超常時性金属酸化物などとの複合粒子（磁性シリカ粒子：直径1~10 μ m）を約0.5~5Mの塩化ナトリウムまたは塩化リチウムに懸濁した組成物がある。該組成物を使用することにより、安定した粒子懸濁液の供給および磁性シリカ粒子の自動分注が可能となるため、核酸の自動抽出機などに応用可能である。

【0025】また、本発明はシリカ粒子組成物を分注器具または分注機器を用いて、試薬容器から反応容器となる試験管、マイクロチューブまたはプレート等へ分注する。分注器具としては、メスピペット、マイクロピペッターおよびシリンジなどが考えられる。分注機器としては、シリンダーを用いたピペットタイプの分注機およびポンプを用いた加圧式分注機などが考えられる。また、分注方法としては、懸濁された溶液をシリンダーを介してチップ内に吸入し分注する方法および管を通してポンプ等の圧力を利用してノズルより押し出し分注する方法などが考えられる。

【0026】さらに、本発明の核酸またはタンパク質抽出用試薬キットは上記シリカ粒子組成物、さらに核酸またはタンパク質を容易に吸着させるための吸着用試薬粒子に吸着した非特異的成分を洗浄する洗浄用試薬および粒子表面から核酸またはタンパク質を溶出するための溶出用試薬を含む。吸着用試薬としては、例えば、クアニジン塩、ヨウ化ナトリウム、ヨウ化カリウム、（イソ）チオンアン酸ナトリウム、尿素またはこれらの混合物などのカオトロピック試薬などが例示される。また、吸着用試薬は、酢酸緩衝液、クエン酸緩衝液、トリス緩衝液などの緩衝剤、エチレンジアミン四酢酸ナトリウム（EDTA）などのキレート剤および、またはエタノールなどの有機溶媒を含有していてもよい。洗浄用試薬は上記トロピックス物質またはエタノールなどの有機溶媒を含有していてもよい。洗浄用試薬は、上記緩衝剤および、またはキレート剤を含有していてもよい。粒子表面から核酸またはタンパク質を溶出する溶出用試薬には、低塩濃度緩衝液、水などを使用する。吸着液、洗浄液および溶出液の成分は、上記以外に用途によって様々な組成を選択することができる。

【0027】また、核酸またはタンパク質を抽出するには、該試薬キットに含まれる試薬を定められた順序で混合、分離および加温等の操作を行う必要がある。例えば、タンパク質および核酸を含む細胞を上記シリカ粒子組成物および核酸またはタンパク質吸着用試薬と混合して、該細胞からタンパク質および核酸を取り出し、該シリカ粒子にタンパク質または核酸を結合させて、残余の成分と分離させ、該シリカ粒子を洗浄した後、該シリカ粒子に結合した核酸またはタンパク質を溶出させること

により、核酸またはタンパク質を抽出する。抽出された核酸は、公知の核酸増幅法を用いて増幅した後、公知の検出法にて検出することができる。本発明の核酸またはタンパク質抽出法は、研究用または臨床診断用の核酸またはタンパク質の調製に適用できる。

【0028】

【実施例】以下に、本発明を実施例より、具体的に説明する。

実施例1 粒子沈降試験

(1) 磁性シリカ粒子懸濁液の調製

磁性シリカ粒子（粒径1～10 μ m、四乙酸鉄粒子30%（w/v）含有、比表面積280m²/g、表面細孔直径2～6nm、細孔容積0.025mL/g；鈴木油脂製）0.4gを下記表1に示される化合物を含む溶液1mLを入れたマイクロチューブの中にて懸濁してシリカ*

*粒子懸濁液を調製した。以下の試薬の調製に用いた試薬は、ナカライテスク社製のものを使用し、水は蒸留水を用いた。

【0029】(2) 沈降試験

(1)にて調製した、磁性シリカ粒子懸濁液それぞれを収容するマイクロチューブを転倒混和して完全に懸濁し、1mLシリンジ（テルモ社製：SS-01T）にて1mLの目盛りまで吸引した。その後、シリンジを試験管立てに固定し、5分後に溶液と磁性シリカ粒子との界面を高さ、すなわち、粒子が何mLのラインまで沈降したかを測定した。測定は2度行い、平均した界面の高さを0分の界面の高さで割ったものを分散率とした。その結果を下記表1に示す

【0030】

【表1】

磁性シリカ粒子懸濁液を5分間静置した時の分散率

試料	分散率
5M塩化ナトリウム溶液	0.95
5Mヨウ化ナトリウム溶液	0.99
5M塩化リチウム溶液	0.97
4M塩化リチウム溶液	0.94
3M塩化リチウム溶液	0.94
2M塩化リチウム溶液	0.94
1M塩化リチウム溶液	0.93
0.5M塩化リチウム溶液	0.90
0.2M塩化リチウム溶液	0.88
5M酢酸アンモニウム溶液	0.96
2M塩化マグネシウム溶液	0.95
2M塩化カリウム溶液	0.85
5Mグアニジン塩酸塩溶液	0.89
50%（w/v）サッカロース溶液	1.00
40%（w/v）サッカロース溶液	0.91
25%（w/v）サッカロース溶液	0.83
50%（w/v）ソルビトール溶液	0.99
50%（w/v）グリセリン溶液	0.88
蒸留水	0.70

【0031】表1から明らかなように、塩化ナトリウム、ヨウ化ナトリウム、塩化リチウム、塩化アンモニウム、塩化マグネシウムなどの塩溶液またはサッカロース、ソルビトールなどの糖類の溶液に、高い粒子分散効果が認められた。これら溶液においては、蒸留水懸濁液が5分後に70%まで界面が下降したのに対して、90%以上の界面が保たれていた。この傾向は、これら溶液濃度が濃いほど顕著であり、5Mの塩化ナトリウム溶液、ヨウ化ナトリウム溶液、塩化リチウム溶液、塩化アンモニウム溶液および2Mの塩化マグネシウム溶液においては、0.95以上の分散率が得られることが分かった。

【0032】塩の濃度については塩化リチウムにおいては、0.5M以上にて顕著であり、0.5Mにおいても、明らかに比重の高い2Mの塩化カリウム溶液よりも分散性が高いことがわかった。また、糖類溶液の濃度についてはサッカロースにおいて、40%（w/v）以上50

の濃度において顕著であった。これらのことから、粒子の分散性を高める効果は、溶液中に溶解している物質の種類およびその濃度に大きく左右されることが明らかとなった。

【0033】また、5分より後の粒子の分散率を5Mの塩化リチウム溶液、蒸留水について比較したところ、30分後においても顕著な差がみられ、蒸留水懸濁液においては分散率が0.4以下であったのに対し、5M塩化リチウム懸濁液においては0.6以上であった（図1）。

【0034】実施例2 分注試験

(1) 粒子懸濁液の調製

実施例1と同様な方法に従い、磁性シリカ粒子懸濁液（5M塩化リチウム溶液、蒸留水）を2mL調製した。

【0035】2. 5mL容量のコンビチップ（エッペンドルフ社製）を装着した連続分注ピペット（エッペンドルフ社製）に（1）にて、既に調製してある5M塩化リ

チウムおよび蒸留水の磁性シリカ粒子懸濁液を1.2m1吸引し、あらかじめ秤量したマイクロチューブに50 μ 1づつ、5秒毎に19本連続分注を行った。分注する際、分注機は床面に対して垂直になるように行った。分注終了後、分注量がほぼ正確であることを確認し、分注されたマイクロチューブを秤量し、あらかじめ秤量しておいた値を差し引いて分注された粒子溶液の重量を算出した。

【0036】その結果を図2に示す。図2から明らかなように、5M塩化リチウムに懸濁した磁性シリカ粒子を分注したものは、蒸留水に懸濁したものに比べ、安定した数値が得られることが分かった。塩化リチウム懸濁液と蒸留水懸濁液の分注重量のCV(%) (標準偏差/平均値 $\times 100$)はそれぞれ0.94%及び、3.26%であった。このばらつきは、分注された懸濁液中に含まれる磁性粒子の濃度に起因していると推察される。

【0037】蒸留水懸濁液を連続分注した場合、最初から13番目までの分注において、その分注された液の重

量が徐々に増加し、13番目以降、徐々に減少する傾向がみられた。この現象は、実施例1の結果からも容易に予測可能であり、時間を経るに従って、粒子懸濁液に濃度勾配が生じていたことが予想される。よって、本発明の方法を用いることにより、均一なシリカ懸濁液を連続的に分注することが可能になることが示された。

【0038】

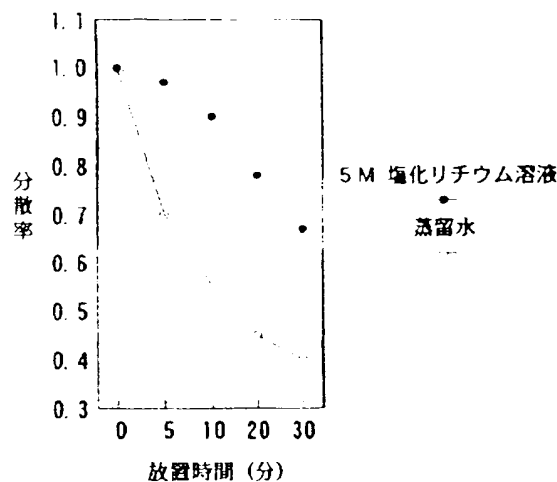
【発明の効果】本発明により、比重の重いシリカ粒子を水溶液中に分散させておくことが容易に可能となる。また、本発明のシリカ粒子組成物は、自動分注器を利用した核酸抽出装置などに応用可能である。この方法を核酸またはタンパク質の精製に応用することにより、従来法に比べ、再現性のある確実な抽出結果が得られる。

【図面の簡単な説明】

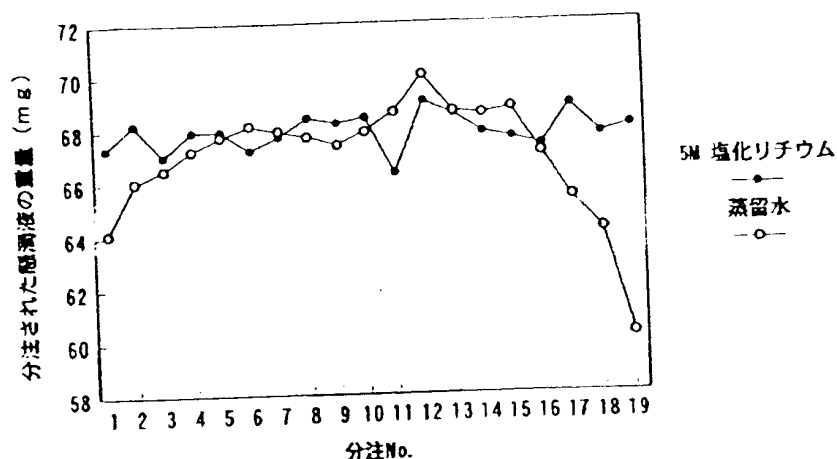
【図1】磁性シリカ粒子懸濁液の分散率と放置時間との関係を示す図である。

【図2】磁性シリカ粒子懸濁液を連続分注した時の、分注重量の変化を示す図である。

【図1】



【図2】



【手続補正書】

【提出日】平成9年6月2日

【手続補正1】

【補正対象書類名】明細書

【補正対象項目名】特許請求の範囲

【補正方法】変更

【補正内容】

【特許請求の範囲】

【請求項1】 シリカ粒子が、少なくとも0.5Mである無機塩または有機塩の塩溶液および または少なくとも40(w/v)%である単糖類、多糖類または糖アルコールの糖類溶液に懸濁されていることを特徴とする核酸またはタンパク質抽出用シリカ粒子組成物。

【請求項2】 シリカ粒子が、二酸化珪素結晶、二酸化珪素結晶以外の珪素酸化物、珪藻土、ガラス粉末、化学修飾シリカまたはシリカと超常磁性金属酸化物との複合体である請求項1に記載のシリカ粒子組成物。

【請求項3】 無機塩または有機塩溶液が、アルカリ金属、アルカリ土類金属またはアンモニアのハロゲン化物、酢酸塩またはチオシアン酸塩を含む溶液である請求項1記載のシリカ粒子組成物。

【請求項4】 無機塩または有機塩溶液が、塩化ナトリウム、ヨウ化ナトリウム、塩化リチウム、ヨウ化リチウム、塩化マグネシウム、酢酸アンモニウムおよびチオシアン酸ナトリウムよりなる群から選択された塩溶液である請求項1に記載のシリカ粒子組成物。

【請求項5】 糖類溶液がグルコース、サッカロース、ソルビトールおよびマンニトールよりなる群から選択された糖類溶液である請求項1に記載のシリカ粒子組成物。

【請求項6】 比重が1.0~1.8であって、粒径が

0.1~100μmである二酸化珪素結晶、二酸化珪素結晶以外の珪素酸化物、珪藻土、ガラス粉末、化学修飾シリカまたはシリカと超常磁性金属酸化物との複合体であるシリカ粒子が、少なくとも0.5Mであるアルカリ金属、アルカリ土類金属またはアンモニアのハロゲン化物、酢酸塩またはチオシアン酸塩を含む溶液および または少なくとも40(w/v)%である単糖類、多糖類または糖アルコールの糖類溶液に懸濁されていることを特徴とする核酸またはタンパク質抽出用シリカ粒子組成物。

【請求項7】 分注器具または分注機器を用いて、請求項1~6のいずれか1項記載のシリカ粒子組成物を試薬容器から反応容器へ分注することを特徴とするシリカ粒子溶液を分注する方法。

【請求項8】 請求項1~6のいずれか1項記載のシリカ粒子組成物、吸着用試薬、洗浄用試薬および溶出用試薬を含む核酸またはタンパク質抽出用試薬キット。

【請求項9】 吸着用試薬が、カオトロピックス試薬、緩衝液および必要により有機溶媒を含む請求項9記載の核酸またはタンパク質抽出用試薬キット。

【請求項10】 タンパク質および核酸を含む細胞を請求項1~6のいずれか1項記載のシリカ粒子組成物および吸着用試薬と混合して、該細胞からタンパク質および核酸を取り出し、該シリカ粒子に核酸またはタンパク質を結合させて、残余の成分と分離させ、該シリカ粒子を洗浄した後、該シリカ粒子に結合した核酸またはタンパク質を溶出させることを特徴とする核酸またはタンパク質抽出法。

【手続補正2】

【補正対象書類名】明細書

【補正対象項目名】0011

【補正方法】変更

【補正内容】

【0011】これらの成分より構成されるシリカ粒子は、水よりも比重が重く、水に懸濁すると沈降し、容器の底に固いペレットを成形する性質があり、長い間、水

溶液中に懸濁状態にしておくことが一般的に困難なものである。特に、分子生物学の分野で用いられている核酸吸着などに使用されている粒子は、直径が約0.1～100 μm 程度のもが多く、それらの多くはこのような性質を有している

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